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Intercolony Coordination of Zooid Behavior and a New Class of Pore Plates in a Marine Bryozoan

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Abstract. This paper describes a mixed allorecognition interaction between adjoining colonies of the encrusting cheilostome bryozoan *Membranipora membranacea,* in which characteristics of both intercolony fusion and intercolony rejection occur simultaneously. Intercolony coordination of zooid behavior was assayed by applying electrical stimuli to one colony of a colony pair while observing the behavior of the adjoining colony. Retraction of feeding structures (lophophores) by the unstimulated colony indicated intercolony coordination of behavior. Naturally occurring and artificially created pairs of genotypically identical and genotypically distinct colonies were examined. Additionally, colony borders were examined for the presence of pore plates, structures that physiologically link zooids within colonies. Contact between genetically identical colonies (isocontact) always resulted in a characteristic border morphology, characteristic pore plates, and intercolony coordination of zooid behavior. Contact between genotypically distinct colonies (allocontact) always resulted in a characteristic border morphology and in the formation of characteristic pore plates of a type never before described. However, only colonies that were young when they first came into contact showed coordinated behavior. Intercolony coordination of zooid behavior is probably the result of neural connections made through pore plates. Intercolony behavioral coordination between young genotypically distinct colonies is peculiar, because the colonies simultaneously show characteristics of physiological integration (coordinated behavior) and tissue rejection (borders and pore plates characteristic of contact between genetically distinct tissues). This interaction shows that the presence of the morphological characteristics of intercolony rejection does not always imply a lack of physiological integration between colonies.

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Introduction

Colonial marine invertebrates such as sponges, cnidarians, bryozoans, and ascidians are capable of indeterminate asexual growth. As a result, contact between conspecific and heterospecific colonies is extremely common on most marine hard substrata where space is limiting (Dayton, 1971; Stebbing, 1973a; Jackson, 1977; Osman, 1977). Many of these colonial invertebrates have highly discriminating immune systems capable of allorecognition—the ability to distinguish between genetically identical and genetically distinct tissue (for review see Grosberg, 1988). If genotypically identical, or closely related (*e.g.*, sibling) colonies come into contact, they commonly fuse into a single colony. If genotypically distinct colonies come into contact, tissue rejection typically follows, and fusion does not occur (Sabbadin, 1982; Scofield *et al.*, 1982; Chaney, 1983; Rinkevich and Loya, 1983a; Shenk and Buss, 1991).

Recent work describing allorecognition responses of colonial marine invertebrates has revealed a diversity of interactions ranging from intercolony fusion to intercolony rejection. Colonies of the hydroid *Hydractinia symbiolongicarpus* may fuse permanently, fuse and then later reject, or reject with the subsequent production of aggressive hyperplasitic stolons (Buss and Grosberg, 1990; Shenk and Buss, 1991). In ascidians, allorecognition responses include permanent fusion, fusion followed by separation, fusion followed by complete resorption of one colony, rejection with little further interaction, and rejection with necrosis of the tissues of one or both colonies (Koyama and Watanabe, 1982; Scofield and Nagashima, 1983; Rinkevich and Weissman, 1987, 1989).

All of the above examples involve either different intensities of rejection or a temporal separation between fusion and rejection. This paper describes a mixed interaction between colonies of the encrusting cheilostome

bryozoan *Membranipora membranacea* involving simultaneous evidence of physiological fusion and tissue rejection. Zooids within bryozoan colonies are physiologically integrated through a nerve net that traverses the calcified zooidal walls through pore plates (Thorpe *et al.*, 1975; Lutaud, 1977, 1979), distinctive structures in the zooidal wall where there is a concentration of several open pores (Silen, 1944; Banta, 1969). The most obvious display of physiological integration of zooids within a bryozoan colony is the coordination of the lophophore retraction response. In response to a localized disturbance to one or a few zooids, all of the zooids within a colony simultaneously retract their feeding structures (lophophores). I have observed that when genotypically distinct colonies of *M. membranacea* come into contact, intercolony coordination of lophophore retraction is frequently observed. Yet, the intercolony borders of these same colonies show no morphological characteristics of fusion.

Because of the mixed nature of this interaction, I will avoid the terms fusion and rejection. Fusion in bryozoans is commonly associated with physiological integration (Stebbing, 1973b; Humphries, 1979; Nielsen, 1981; Chaney, 1983); consequently, the term fusion could also be applied to colonies that show physiological integration, but lack any morphological characteristics of fusion. To avoid this ambiguity, I will refer to contact between genetically distinct tissues as "allocontact", and I will refer to contact between genetically identical tissues as "isocontact." The physiological consequences and morphology of these interactions can then be described separately.

M. membranacea occurs naturally in dense monospecific populations where contact between conspecifics is extremely common, if not unavoidable. Larvae of *M. membranacea* disperse in the plankton for up to four weeks (Yoshioka, 1982), thus naturally settled adjoining colonies are unlikely to be siblings. Consequently, the majority of intercolony interactions are between unrelated colonies. However, contact between genotypically identical tissues occasionally occurs when a single colony grows into contact with itself after either growing around some object or fusion resulting from damage to the colony (pers. obs.). I will (1) examine how intercolony coordination is related to the size and age at which genotypically distinct colonies first come into contact, (2) compare the morphology of the borders between genotypically distinct colonies to those between genotypically identical colonies, and (3) examine both types of borders for pore plates that could facilitate intercolony coordination of zooid behavior.

Materials and Methods

Animal collection

Research was conducted at Friday Harbor Laboratories (FHL), San Juan Island, Washington, and at the University of California, Los Angeles. At FHL, colonies of *Membranipora membranacea* that had settled on black acrylic panels suspended from the FHL dock, as well as colonies collected from the field, were used in this study. Colonies were collected from the field by haphazardly selecting bryozoan-encrusted blades of the kelp *Laminaria* sp. from Turn Island and transporting them back to FHL where the kelp blades were hung from the FHL dock. In California, *M. membranacea* colonies were collected from kelp beds off the coast of Malibu, California. Bryozoan encrusted blades of the kelp *Macrocystis pyrifera* were haphazardly removed from the upper parts of kelp fronds on, and just below, the surface of the water. Blades were then transported back to the laboratory where they were maintained in a recirculating seawater system.

Intercolony coordination of lophophore retraction

To ensure that a given intercolony border was between two colonies descended from different larvae rather than previously separated parts of a colony decended from a single larva, I used only colony pairs for which I could locate both ancestrulae. The ancestrula is a pair of morphologically distinct zooids that develop from the larva after settlement and metamorphosis (Fig. 1A). Ancestrular zooids are easily distinguished from younger asexually produced zooids because they are rounder, more heavily calcified, and together are distinctively heart shaped (Fig. 1B). Unless indicated otherwise, whenever I mention colony pairs, I will be referring to pairs of colonies descended from separate larvae.

To test for intercolony coordination of lophophore retraction, I stimulated colony pairs electrically. A stimulus was applied to one of the two colonies. A colony-wide lophophore retraction response in the adjoining unstimulated colony was used as an indication of intercolony behavioral coordination. Electrical stimuli were applied with an electrode placed on the surface of the colony. All stimuli were at, or just above, the threshold stimulus (a single square pulse between 5 and 10 volts for 5–10 ms) required to elicit a colony-wide lophophore retraction response. In addition to electrical stimuli, mechanical stimuli were applied to pairs of very small colonies (less than 10 mm^2) to eliminate the possibility that intercolony coordination was an artifact resulting from electrical conduction of the stimulus through the water or across the colony surface. Mechanical stimuli were applied by lightly touching a dissecting needle to one of the colonies on the edge opposite the intercolony border.

To determine whether the non-stimulated colony of a pair of behaviorally coordinated colonies was responding to the physical retraction of the lophophores of the adjoining colony, I retested 20 coordinated colony pairs after first making a fine cut with a razor blade along the border

between the adjoining colonies. Cuts were made so that no lophophores along the intercolony borders were damaged.

To determine whether coordination was bidirectional, a stimulus was applied to one colony of a pair until I had obtained 20 behaviorally coordinated and 20 non-coordinated pairs. A second stimulus was then applied to the other colony of each pair.

The frequency of intercolony behavioral coordination in a natural population of *M. membranacea* was measured at Friday Harbor by sampling three blades of *Laminaria*. Both sides of 5 × 10 cm rectangles were censused 5 cm from each edge of the blade at 25, 50, 75, 100, and 125 cm from the base of each blade (where the stipe meets the blade). Each colony was recorded as being solitary or in contact with other colonies. If a colony was in contact with another colony, it was tested for intercolony coordination of lophophore retraction. In all, 1301 colonies were sampled.

Intercolony coordination and size at first contact

To determine the relationship between colony size at first contact and intercolony coordination, 92 pairs of *M. membranacea* colonies were cultured on black acrylic panels in Friday Harbor. Panels were cleared at least once a week of all other organisms. Each colony monitored was in contact with only one other colony. The size of each colony at the time of first intercolony contact was determined by tracing each colony on acetate paper and calculating the area of the tracing using a video-integrated image analysis system. Following contact, all colony pairs were tested for intercolony coordination of lophophore retraction one to three times each week for five weeks.

Additional data on the relationship between intercolony coordination and colony size at first contact were obtained for *M. membranacea* colonies in California. Densities of *M. membranacea* in California tend to be higher than in Friday Harbor (pers. obs.). As a result, data could be obtained for adjoining colonies that were typically smaller at first contact than those observed in Friday Harbor. In all, 230 colony pairs were selected from 10 different *Macrocystis* blades. Colonies were selected to give a maximum range of values for size at first contact. Because of high colony density, colony "pairs" were sometimes in serial contact with other colonies (forming linear groups of 3, 4, or more colonies). However, no colony was ever in contact with more than two other colonies, and a single colony was never used more than once. Colonies were examined using a dissecting microscope, and all measurements were made with an ocular micrometer.

Because I was unable to culture colonies in California, direct measurements of colony size at first contact were not possible. Instead, I estimated colony size at first contact by measuring the intercolony ancestrula distance (Fig. 3). Because the ancestrula marks the site of larval settlement and metamorphosis, I assumed that the distance between the ancestrulae of two colonies would be directly correlated to the size of the colonies at first contact. Additionally, it seemed likely that colonies would not become coordinated immediately upon contact, but would instead require a period of time for the formation of intercolony physiological connections. Consequently, for each colony pair I also estimated how long colonies had been in contact by measuring the intercolony border length (Fig. 3). Because the length of the border between colonies increases as both colonies grow, I assumed that the length of the intercolony border would be directly correlated to how long the colonies had been in contact. After making these measurements, colonies were tested for intercolony coordination of lophophore retraction.

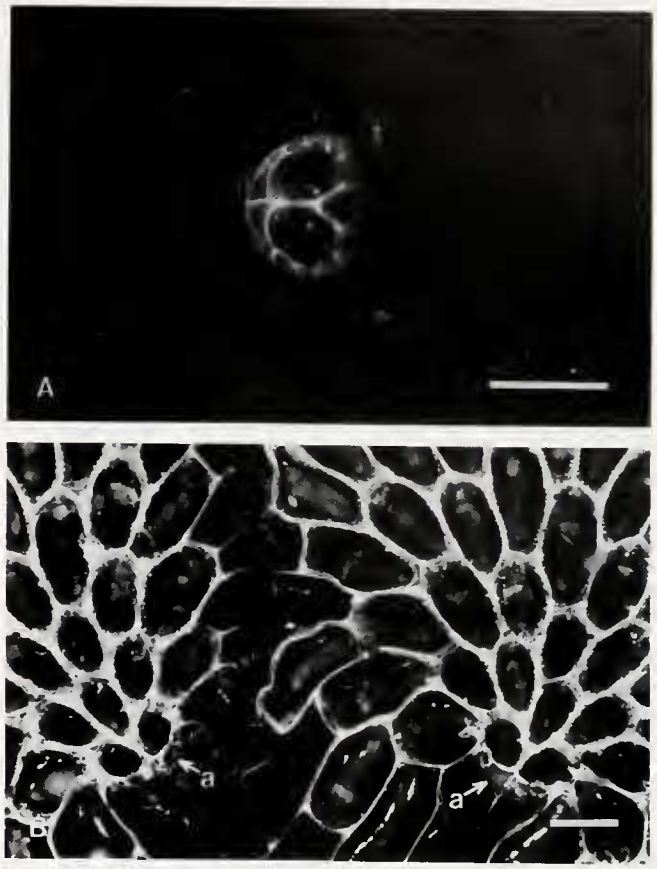

Figure 1. A. The founding ancestrula of a colony shortly after larval settlement and metamorphosis. B. Ancestrulae and intercolony border of a pair of colonies that have grown into contact. Small bubble-like structures visible along the intercolony border are allocontact pore plates. Abbreviation: a, ancestrula. Size bars = 0.5 mm.

Transplant experiment

Although unlikely, I cannot be sure that naturally settled adjoining colonies are not genetically similar siblings

that have settled in close proximity. To determine whether behavioral coordination can occur between colonies that are clearly not siblings, I paired *M. membranacea* colonies from Turn Island with colonies from Rocky Point, San Juan Island, a site approximately 10 miles northwest from Turn Island. Bryozoan encrusted blades of the red alga *Iridea* were collected from the two sites. I removed 48 small colonies (<25 mm^2) from the algal blades by gently stretching the blade until the colony detached. Twenty-four colony pairs, each consisting of one colony from Turn Island and one colony from Rocky Point, were then placed on acrylic panels. After 24 h, colonies had attached to the panels that were subsequently suspended below the FHL docks. Following contact, all colony pairs were tested for intercolony coordination of lophophore retraction twice each week for four weeks.

Size reduction experiment: allocontact and isocontact

To distinguish the effects of colony age from those of colony size and to establish unambiguous examples of isocontact between completely separated parts of a single colony, I reduced large colonies growing on acrylic panels at Friday Harbor to pairs of smaller subcolonies. Using a razor blade to cleanly cut a square of the appropriate size in the colony, I created pairs of either small or large square subcolonies that were 16 mm^2 or 100 mm^2, respectively. All other parts of the colony were then scraped off the panel with a small spatula. A 1-mm strip of space was also scraped between each colony pair.

Allocontact pairs were created by making subcolonies on both sides of the intercolony border between pairs of non-coordinated colonies (after testing for behavioral coordination). In all, eight small and seven large allocontact pairs were established. Isocontact pairs were created by reducing single colonies into two smaller subcolonies. In all, seven small and eight large isocontact pairs were established. In addition to providing an unambiguous example of isocontact, this latter treatment also served as a control for possible effects of damage on the establishment of behavioral coordination, because adjoining parts of a single colony should become physiologically integrated when they meet. Regeneration and growth of the cut borders was rapid; all colony pairs had grown back into contact in approximately a week. After subcolonies had grown into contact, I tested for intercolony behavioral coordination.

Pore plates

A scanning electron microscope was used to examine isocontact borders (n = 2) and allocontact borders of coordinated (n = 2) and non-coordinated (n = 2) colony pairs for the presence of pore plates. Colonies growing on *Laminaria* were collected at Friday Harbor. For isocontact, only single colonies that had grown around some object and back into contact with itself were used; for allocontact, only colonies with both ancestrulae present were used. Colonies were prepared by dissolving away the tissues of colonies in 2.5% sodium hypochlorite for 12 h to expose the calcium carbonate skeleton.

Isocontact and allocontact borders of naturally occurring colonies were also examined histologically for pore plates. I examined isocontact borders (n = 3) and allocontact borders between behaviorally coordinated (n = 6) and non-coordinated (n = 6) colony pairs collected in California. Approximately 2–3 mm long sections of borders, along with the kelp substrate, were removed with a razor blade. Samples were first fixed in 3% glutaraldehyde in 0.1 *M* sodium cacodylate buffer, pH 7.4 for 1 h, and then in 4% osmium in 0.1 *M* sodium cacodylate buffer for an additional hour. Samples were then dehydrated in a graded series of ethanol dilutions, treated with propylene oxide, and infiltrated overnight in Medcast low viscosity embedding medium. After polymerizing overnight at 70°C, samples were sectioned (approximately 3 μ thick) and viewed using a light microscope.

Results

Intercolony coordination of lophophore retraction

A cut between behaviorally coordinated colony pairs always completely eliminated intercolony coordination of lophophore retraction. Thus, colonies were not responding to the physical disturbance created by the retraction of the lophophores of adjoining colonies.

For all behaviorally coordinated colony pairs tested, intercolony coordination was always bidirectional. Stimulation of either colony resulted in a colony-wide lophophore retraction response in the non-stimulated colony. Unstimulated colonies of non-coordinated pairs always failed to respond regardless of which colony was stimulated. No colony pairs were found in which information flow was unidirectional.

Intercolony coordination of behavior is frequently observed in natural populations. Of the 1301 colonies sampled from *Laminaria* blades, 568 (44%) were in contact with another colony. Of these, 408 (72%) were behaviorally coordinated with at least one neighbor.

Intercolony coordination and size at first contact

Intercolony coordination of zooids was observed most frequently when two colonies were small at the time of first contact (Fig. 2). When the areas of each colony in a pair at the time of first contact were summed, the combined area of colony pairs with coordinated behavior (n = 15; mean = 1.02 cm^2, S.D. = 1.51) was significantly smaller than the combined area of colony pairs that were

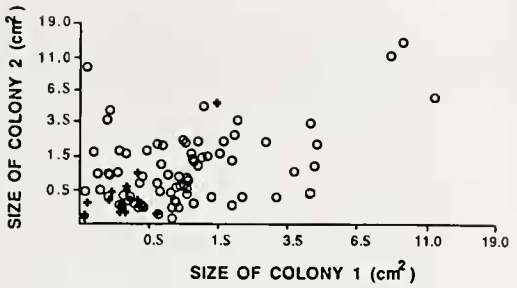

Figure 2. Colony size at first contact and intercolony behavior for 92 colony pairs cultured on acrylic panels. Each colony is plotted by the size of each colony in the pair at the time of initial intercolony contact. One colony of each pair was arbitrarily designated colony 1 and the other colony 2; + = coordinated colony pair, o = non-coordinated colony pair. Note that data are plotted on a logarithmic scale.

not coordinated (n = 77; mean = 3.05 cm^2, S.D. = 3.76; t-test of ln transformed data, $P < 0.001$). In all pairs that became behaviorally coordinated, there was a short period (approximately a week) following initial contact during which colonies were not behaviorally coordinated. Coordinated behavior of the one outlying pair in Figure 2 was observed on only a single occasion, suggesting either human error or that the pair was anomalous.

There was a significant relationship between intercolony coordination and both the estimated size at first contact (intercolony ancestrula distance; $\chi^2 = 46.82$, $P < 0.0001$) and the estimated length of time in contact (intercolony border length; $\chi^2 = 22.22$, $P < 0.0001$) for colony pairs from California. Data were analyzed using multiple logistic regression with intercolony ancestrula distance and intercolony border length as independent variables and behavior, coordinated or not coordinated, as the binary dependent variable. As the estimate of colony size at first contact increased, the probability of intercolony coordination decreased (Fig. 3). Although many colony pairs with small intercolony ancestrula distances (*e.g.* < 7 mm) were not coordinated, the majority of these also had small intercolony border lengths relative to the coordinated colony pairs.

Transplant experiments

Of the 24 colony pairs composed of one colony from Turn Island and one colony from Rocky Point, 20 remained attached to the acrylic panels and grew into contact. Of these, 13 (65%) showed intercolony coordination of zooid behavior within two weeks, thus demonstrating that coordinated behavior can occur between colonies that are clearly not related. For all colony pairs that became coordinated, there was a brief period (approximately one week) following initial intercolony contact when colonies were not coordinated.

Size reduction experiment: allocontact and isocontact

In the size reduction experiment, none of the allocontact colony pairs became behaviorally coordinated, regardless of size. Thus, if colonies are genotypically distinct, age rather than size appears to be the most important factor determining whether intercolony coordination occurs. All isocontact colony pairs did become behaviorally coordinated, regardless of size.

Isocontact borders were morphologically distinct from allocontact borders. Isocontact borders were straight and fully calcified, and zooids distal to the area of first contact aligned to form a single growing edge (Fig. 4A). Allocontact borders were clearly distinct from isocontact borders. Allocontact borders were not as straight as isocontact borders and were uncalcified or only lightly calcified, and each colony maintained a separate growing edge (Fig. 4B).

Pore plates

All isocontact and all allocontact borders (both from coordinated and non-coordinated colony pairs) contained structures (Figs. 5, 6) that clearly resemble the previously described bryozoan pore plates, transverse pore plates, lateral pore plates, and fusion pore plates (Silen, 1944; Banta, 1969; Chaney, 1983). Transverse and lateral pore plates are located respectively in the transverse and lateral zooidal walls that separate adjoining zooids within the same colony (Figs. 5A, B; 6A). Fusion pore plates are found in the walls between two colonies that have fused into a single colony (Chaney, 1983). Pore plates are round (lateral and fusion pore plates) to elliptical (transverse pore

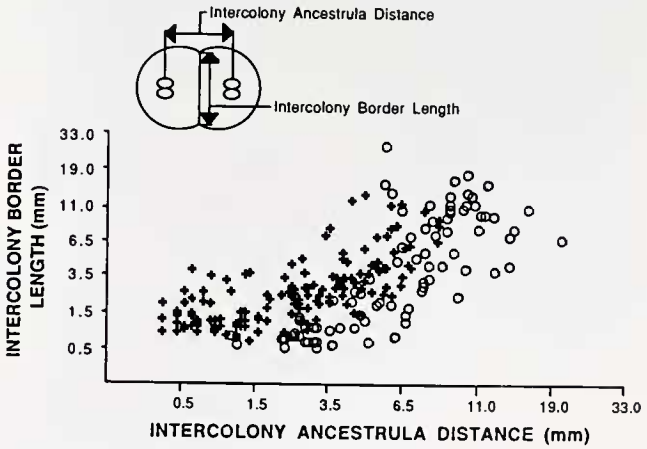

Figure 3. Intercolonial ancestrula distance, intercolonial border length and intercolonial behavior for 230 colony pairs from *Macrocystis* blades. For each colony pair intercolony ancestrula distance (estimate of size at first contact) is plotted against intercolony border length (estimate of time since first contact); + = coordinated colony pair, o = non-coordinated colony pair. Note that data are plotted on a logarithmic scale.

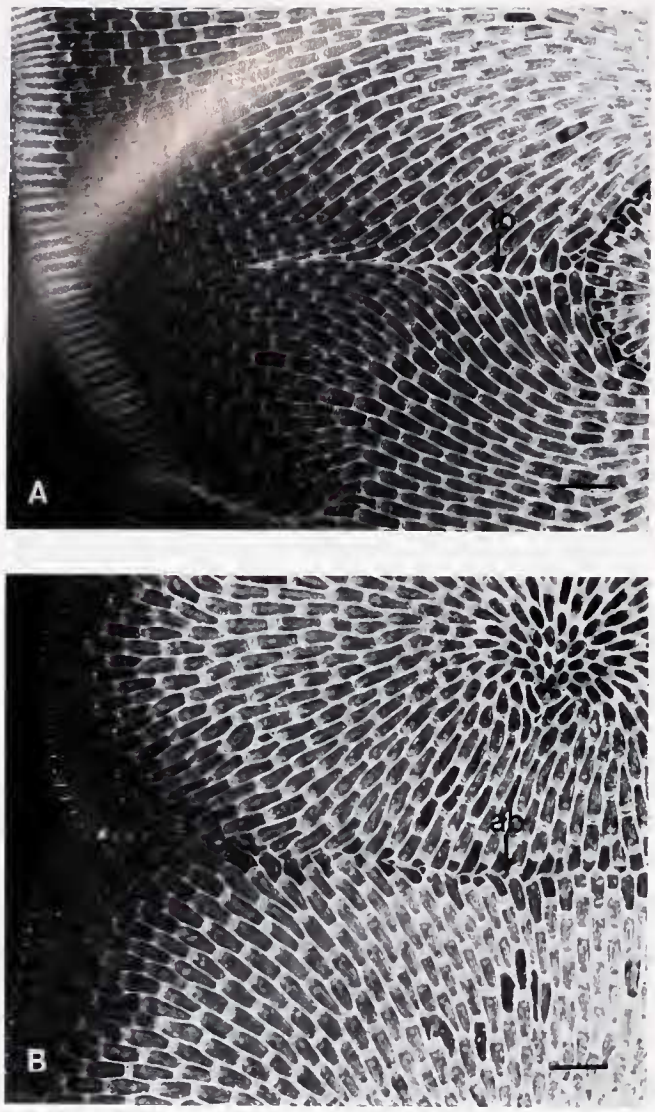

Figure 4. A. Isocontact border between genotypically identical tissues of a colony that has grown around another colony and back into contact with itself. B. Allocontact border between genotypically distinct colonies. Abbreviations: ab, allocontact border; ib, isocontact border; Size bars = 1.0 mm.

plates) in shape and slightly raised to form a perforated calcium carbonate dome or lens, the base of which is attached to the zooidal wall (Fig. 5A–C).

Pore plates found in isocontact borders, herein referred to as "isocontact pore plates" (Figs. 5C, 6B), were similar to lateral pore plates in that they consisted of a single round perforated dome. However, whereas lateral pore plates tended be of a uniform size and regularly spaced in lateral walls, isocontact pore plates were variable in size and occurred irregularly, occasionally in groups, in the walls formed between genotypically identical colonies.

Pore plates found in allocontact borders, herein referred to as "allocontact pore plates" (Figs. 5D; 6C, D) were found in the borders between both coordinated and non-coordinated colonies. Whereas all previously described pore plates consist of a single perforated calcium carbonate dome, allocontact pore plates were composed of two perforated calcium carbonate domes placed base to base forming a single sphere embedded in the intercolony border. Allocontact pore plates also differed from other pore plates in that they generally had three or fewer pores. In contrast, other types of pore plates generally had four or more pores. There were no obvious morphological differences between allocontact pore plates of coordinated and non-coordinated colonies.

Discussion

The results of this study show that allorecognition responses following contact between colonies of the bryozoan *Membranipora membranacea* vary depending on the genetic similarity and age of interacting colonies. Contact between genetically identical colonies is always characterized by an isocontact border, isocontact pore plates, and coordinated behavior of zooids. Contact between genotypically distinct colonies is always characterized by allocontact borders and allocontact pore plates. However, only colonies that are young when they first come into contact, show coordinated behavior.

Intercolony coordinated behavior appears to be the result of intercolony neural integration. Thorpe *et al.* (1975) demonstrated the presence of electrical signals that conducted across colonies of *M. membranacea* at the same rate as the spread of lophophore retractions. Electrical signals similar to those described by Thorpe *et al.* (1975) have been found to pass between behaviorally coordinated colonies but not between non-coordinated colonies (Shapiro and Mackie, unpub. data), providing direct evidence of intercolony neural linkage.

The presence of pore plates provides morphological evidence for intercolony neural linkage. The time required for the formation of isocontact or allocontact pore plates following initial intercolony contact would explain why colonies did not become coordinated immediately upon contact and why colonies with short intercolony border lengths did not show coordinated behavior. However, the presence of allocontact pore plates does not necessarily indicate behavioral coordination because allocontact pore plates were also found between non-coordinated colony pairs. Thus, there may be morphological differences on a finer scale (*e.g.*, presence or absence of functional nerves) between the allocontact pore plates of behaviorally coordinated and non-coordinated colonies.

Allocontact pore plates represent a new, morphologically distinct class of pore plates never before described in the Bryozoa. This is the first time pore plates between unrelated bryozoan colonies have been described. Chaney

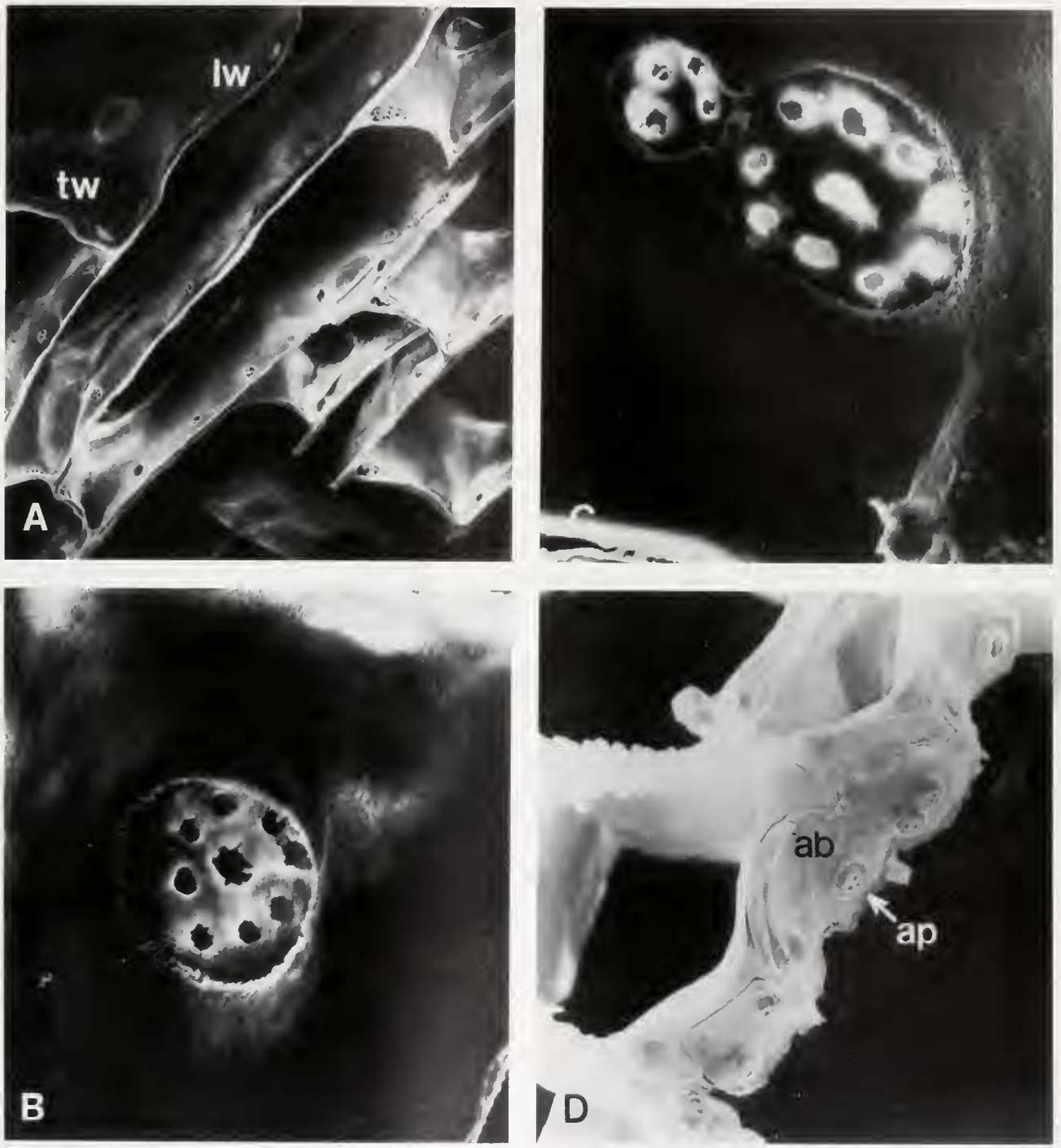

Figure 5. Scanning electron micrographs of the different types of pore plates found in *Membranipora membranacea*. A. Basal view of calcified zooidal walls showing transverse and lateral pore plates between zooids within a colony. (100×). B. Lateral pore plate between zooids within a colony (500×). C. Isocontact pore plates (500×). D. Allocontact border showing allocontact pore plates (100×). Abbreviations: ab, allocontact border; ap, allocontact pore plate; lw, lateral wall; tw, transverse wall.

(1983) examined the borders between unrelated colonies of the cheilostome bryozoan *Thalamoporella californica*, but found no evidence of pore plates. However, Chaney (1983) did find pore plates between sibling colonies of *T. californica*. These pore plates, which he called fusion pore plates, consisted of a single rather than a double calcium

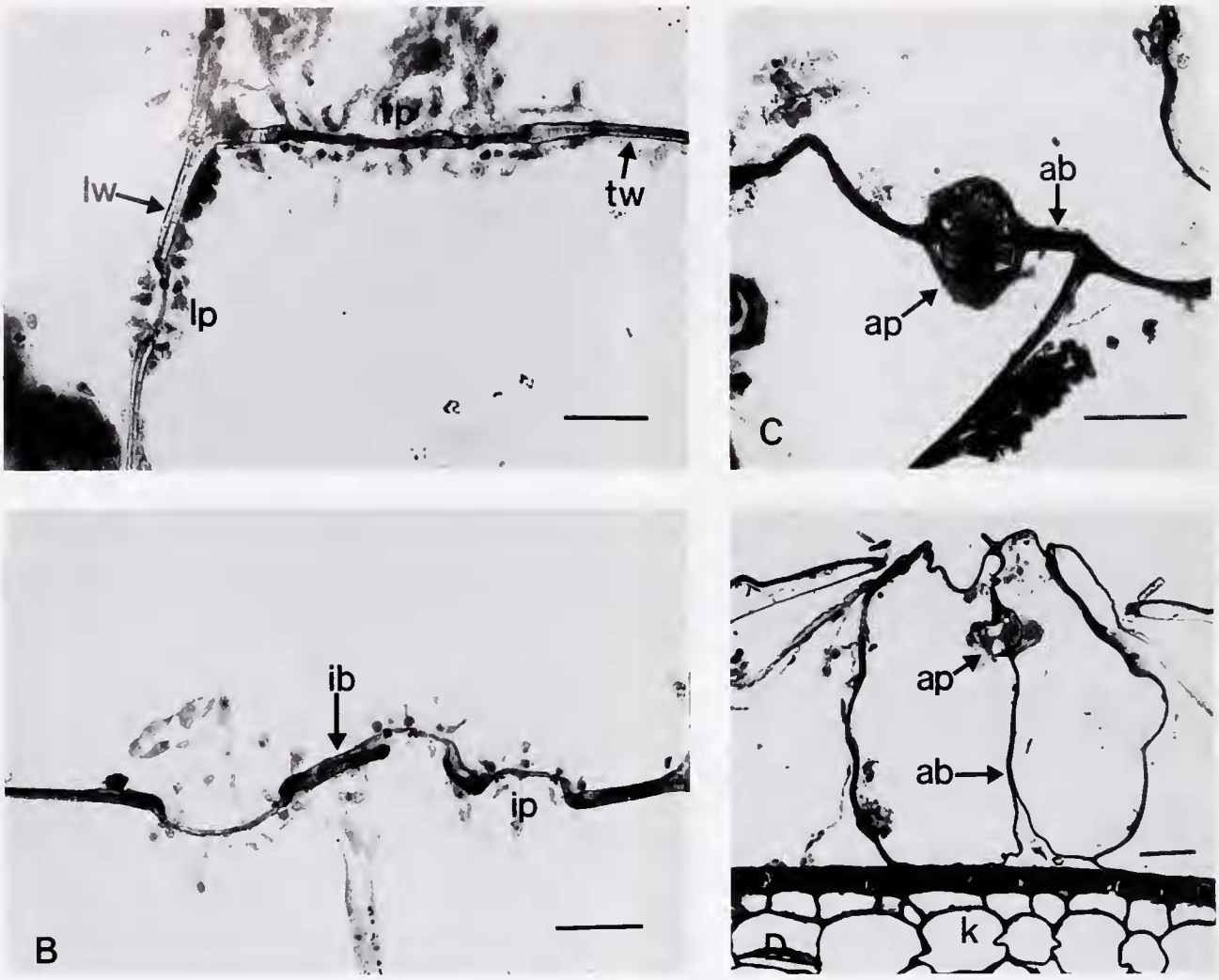

Figure 6. Light micrographs of the different types of pore plates found in *Membranipora membranacea*. Sections A through C were made parallel to the plane of the colony. A. Transverse and lateral pore plates between zooids within a colony. B. Isocontact plates. C. Allocontact plate. D. Section perpendicular to allocontact border and plane of colony showing an allocontact plate. Abbreviations: ab, allocontact border; ap, allocontact plate; ib, isocontact border; ip, isocontact plate; k, kelp; lw, lateral zooidal wall; tp, transverse pore plate; tw, transverse zooidal wall. Size bars = 20 μm.

carbonate dome and thus resemble the isocontact pore plates described in this study and not allocontact pore plates. Additionally, fusion pore plates, like isocontact pore plates, were variable in size and occurred irregularly in the walls formed by contact between two colonies. *T. californica* larvae settle within hours of release from the parental colony (Chaney, 1983), thus indicating the potential for substantial inbreeding in natural populations (Jackson, 1986). Consequently, although sexually produced, sibling colonies may be nearly genetically identical. Thus, fusion pores plates are probably the same as isocontact pore plates, both being characteristic of contact between genetically similar tissues.

It is usually assumed that colony pairs that have the morphological characteristics of fusion are physiologically integrated, and unfused colonies are not (Humphries, 1979; Stebbing, 1973b; Buss, 1982; Chaney, 1983). However, assays for physiological integration are rarely performed (Hidaka, 1985; Rinkevich and Loya, 1983a, b). When Rinkevich and Loya (1983a) used SEM to examine the allocontact borders between colonies of the Red Sea coral *Stylophora pistillata* with the morphological characteristics of fusion, they found that the colonies were not physiologically connected. In contrast, this study has demonstrated that *M. membranacea* colonies with the morphological characteristics of rejection can be physi-

ologically connected. Thus, unless adequate tests are performed, it may not always be safe to use morphological evidence of fusion or rejection to imply the presence or absence of physiological integration.

Allorecognition responses are important in intra- and interspecific interactions. Rejection responses to contact with colonies often result in the induction of aggressive structures used to fight, damage, or surround neighboring colonies (*e.g.,* Ivker, 1972; Francis, 1973; Rinkevich and Loya, 1983a; Sebens and Miles, 1988; Harvell and Padilla, 1990). On the other hand, fusion responses may benefit interacting colonies by increasing competitive ability, increasing fecundity, decreasing probability of mortality, or decreasing age of first reproduction (Buss, 1982). However, it may be erroneous to always associate fusion with cooperation and rejection with aggression. Rinkevich and Weissman (1987, 1989) found that fusion between genotypically distinct ascidian colonies frequently resulted in partial or total resorption of one of the colonies at a cost to both colonies. Thus, in this case fusion is apparently an aggressive interaction. In contrast, the results of the present study indicate the potential for cooperation between colonies that do not appear to have fused.

Intercolony behavioral coordination may be an adaptation that benefits small colonies by reducing the probability of mortality. Mortality of many marine invertebrates, including bryozoans, is size dependent, with small colonies having a higher probability of mortality (Jackson, 1985; Yund and Parker, 1989; Harvell *et al.,* 1990). Coordinated behavior between small *M. membranacea* colonies may benefit each colony by enabling colonies to receive and transmit signals that act as "warnings" of possible sources of damage or mortality. Such cooperative behavior is consistent with theory predicting that cooperation will be more likely to evolve between sessile organisms that interact repeatedly (Axelrod and Hamilton, 1981; Buss, 1981).

It could also be argued that intercolony behavioral coordination is a non-adaptive trait that results from the inability of young colonies to distinguish between genetically identical and genotypically distinct tissues. There are several examples of colonial marine invertebrates that will fuse when young but not when older (*e.g.,* Hidaka, 1985; Shenk and Buss, 1991). It is not known what causes changes in fusibility, although immunological incompetence of young colonies has been suggested (Hidaka, 1985). However, if immature, genotypically distinct colonies of *M. membranacea* were simply treating adjoining colonies as genotypically identical, intercolony borders and pore plates should resemble isocontact borders and isocontact pore plates. Instead, typical allocontact borders and allocontact pore plates were formed between all genotypically distinct behaviorally coordinated colonies implying that colonies had recognized their neighbors as being genotypically distinct.

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